AN ARSENIC-SPECIFIC BIOSENSOR WITH GENETICALLY ENGINEERED *SHEWANELLA ONEIDENSIS* IN A BIOELETROCHEMICAL SYSTEM

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by
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RESEARCH HIGHLIGHTS

(1) We have developed the first bioelectrochemical system (BES) based environmental biosensor with genetically encoded specificity for a toxic metal.

(2) This biosensor employs a genetically engineered *Shewanella oneidensis* strain, which was designed to produce more current in response to arsenic when cultured in a BES.

(3) Our first-generation BES-based biosensor has a response time of approximately 24 h and a detection limit on the order of 100 μM arsenite.

ABSTRACT

Genetically-engineered microbial biosensors have yet to realize commercial success in environmental applications, due in part to difficulties associated with transducing and transmitting traditional bioluminescent information. The use of bioelectrochemical systems (BESs) in biosensing applications allows for a direct electronic output that can be more easily incorporated into devices for remote environmental monitoring. Herein, we describe the first BES-based biosensor with genetically encoded specificity for a toxic metal. By placing an essential element of the metal reduction (Mtr) pathway of *Shewanella oneidensis* MR-1 under the control of an arsenic-sensitive promoter, we have engineered a strain that produces increased current in response to arsenic when inoculated into an BES. When operated as a chemostat with a hydraulic retention time of 7 h, our BES-based biosensor has a response time of 24 h and a lower detection limit on the order of 100 μM arsenite. To heighten the sensor's sensitivity to arsenic, we have begun characterizing an alternative sensing strain with improved translational efficiency. This ability to tune analyte sensitivity—along with the reliability of our continuous assay and the simplicity of the transcriptional circuit required for BES-based biosensing—suggests that similar sensing systems may be readily developed for both environmental deployment and on-line process control.

1. INTRODUCTION

Microbes are thought to be a promising platform for biosensing since their simplicity to cultivate, ability to thrive under adverse conditions, and amenability to modern recombinant DNA technologies suggest that microbial biosensors can offer inexpensive and specific measurement tools (D'Souza, 2001). Past microbial biosensors have been principally developed for environmental applications, since microbes have at their disposal the tools to sense both bioavailability and toxicity (Belkin, 2003). Many of these have relied on the analyte-induced expression of a reporter genes that produce a bioluminescent, fluorescent, or colorimetric signal (Joshi et al., 2009; Ramanathan et al., 1997; Sagi et al., 2003; Wang et al., 2012). Most notably, *Pseudomonas flourescens* HK44 is a well-characterized strain that has been deployed to monitor naphthalene and salicylate bioavailability in soils (Heitzer et al., 1992; King et al., 1990). Despite their popularity in academic literature, however, genetically engineered microbial biosensors have yet to see commercial success in their target environments (Shin, 2011).

One possible explanation for this gap between academia and industry is the difficulty of detecting the expression of common reporter genes—typically related to either luciferase, green fluorescent protein, or β-galactosidase (Lei et al., 2006)—remotely. Because remote monitoring requires neither on-site technicians nor transportation of samples to laboratories, the ability of microbial biosensors to transmit information across distances will likely be central in their penetration of the environmental monitoring market. Consequently, there has been recent interest in the development of deployable bioluminescent biosensors for long-term remote monitoring (Nivens et al., 2004). However, this technology has yet to gain traction—largely due

to the difficulty of creating a device that not only facilitates the transduction and transmission of bioluminescent information, but also provides an adequate environment to maintain engineered microbes for extended periods of time (Bjerketorp et al., 2006).

A promising alternative that may overcome the limitations of more traditional microbial biosensing methods is the use of bioelectrochemical systems (BESs), which take advantage of the ability of microbes to catalyze the reduction of an anode in an electrochemical cell (Friedman et al., 2012b). Most commonly, BES technologies are developed as microbial fuel cells, which are designed to harvest electricity from chemical energy stored in organic waste. Because direct electronic output is provided by BESs, however, this technology is also ideal for many biosensing purposes (TerAvest et al., 2011). While past BES-based biosensors have been designed to detect biological oxygen demand (Kim et al., 2003), carbon source availability (Kim et al., 1999), and general toxicity (Wang et al., 2013), only recently has it been shown that specific chemical signals may be detected by such sensors (Golitsch et al., 2013). Herein, we move beyond this recent proof of principle and describe a genetically encoded biosensor for arsenic that employs a *Shewanella oneidensis* strain growing in a continuous flow BES. The sensing strains are genomic Δ*mtrB* mutants, and are complemented by a plasmid-encoded copy of *mtrB* that is driven by an arsenic-inducible promoter. Because MtrB is essential for electrode reduction (Coursolle et al., 2010), current production is upregulated in response to arsenic.

2. MATERIALS AND METHODS

2.1 Strains and growth conditions

S. oneidensis MR-1 is an environmental isolate first found in Lake Oneida, New York (Myers and Nealson, 1988). We used an MR-1 *mtrB* deletion mutant (Δ*mtrB*) as a basis for the construction of our arsenic-sensing strains (Coursolle and Gralnick, 2012). During experiments, we employed minimal M4 media, which contains—per liter of deionized water—0.221 g K_2HPO_4, 0.099 g KH_2PO_4, 0.168 g $NaHCO_3$, 1.189 g $(NH4)_2SO_4$, 7.305 g NaCl, 1.192 g HEPES; 0.0713 g $CaCl_2$, and 10 mL M4 trace mineral solution. Per liter deionized water, M4 trace mineral solution contains 2.26 g Na_2EDTA, 24.89 g $MgSO_4$·7H2O, 0.029 g $MnSO_4$·$4H_2O$, 0.058 g NaCl, 0.068 g $FeCl_2$, 0.065 g $CoCl_2$, 0.029 g $ZnSO_4$·$7H_2O$, 0.005 g $CuSO_4$·$5H_2O$, 0.35 g H_3BO_3, 0.08 g Na_2MoO_4, 0.119 $NiCl_2$·$6H_2O$, and 0.028 Na_2SeO_4 (Rosenbaum et al., 2010). When appropriate, sodium L-lactate and Fe(III) citrate were added to minimal M4 media at final concentrations of 20 and 10 mM, respectively, to provide a carbon source and electron acceptor.

Escherichia coli DH5α and WM3064 (a diaminopimelic acid (DAP) auxotroph derived from strain β2155; Dehio and Meyer, 1997) were used for routine cloning and conjugation into *Shewanella* strains, respectively. For growth of *E. coli* WM3064, we supplemented all media with 300 μM DAP. All *Escherichia* strains were grown at 37 °C unless otherwise noted; all *Shewanella* strains were grown at either room temperature or 30 °C. All strains were maintained on solid lysogeny broth (LB) medium at 1.5% agar. Overnight cultures were grown in 5 mL volumes of LB—either from single colonies on agar plates or from frozen stocks maintained in glycerol. When appropriate, we added kanamycin at a final concentration of 50 μg/mL to growth media.

2.2 Mutant construction

We used standard molecular biology techniques to construct plasmids containing arsenic-responsive genetic circuits. We obtained BBa_K098994 and BBa_J33201—derived from the genomes of *S. oneidensis* and *E. coli* JM109 (Diorio et al., 1995), respectively—from the Registry of Standard Biological Parts (http://partsregistry.org). BBa_J33201, which contains both an arsenic-inducible promoter (P_{ars}) and its downstream transcriptional regulator (*arsR*), was amplified *via* PCR using a forward primer (ArsR_1) upstream of an EcoRI/XbaI multiple cloning site (MCS), and a reverse primer (ArsR_2) with a 5' extension introducing AscI, SpeI, and PstI cutsites (see Table S1 in Supporting Information for a list of all primers used in this study).

To facilitate the construction of the initial sensing plasmid, the coding region of *mtrB* was amplified from BBa_K098994 using a forward primer (MtrB_1) introducing AscI and BamHI cutsites flanking a ribosomal binding site (RBS) and a reverse primer (MtrB_2) introducing KpnI, SpeI, and PstI cutsites. The AscI and BamHI cutsites were included to expedite possible modifications to the RBS associated with *mtrB*. Upon digesting the resulting PCR products with EcoRI/AscI and AscI/PstI, plasmid pArsR/MtrB_1 was constructed upon the simultaneous ligation of the two fragments into pBBRBB (Addgene plasmid 32549; Vick et al., 2011), a broad host range mobile vector conferring kanamycin resistance, derived from pBBR1MCS-2 (Kovach et al., 1995). A second plasmid (pArsR/MtrB_2) was also constructed—without a BamHI cut site between the RBS and start codon associated with *mtrB—via* the direct digestion of the *mtrB* coding sequence from BBa_K098994. This plasmid was constructed to control for possible changes to translational efficiency with the modified *mtrB* upstream sequence in pArsR/MtrB_1.

Plasmids pArsR/MtrB_1 and pArsR/MtrB_2 were electroporated into electrocompetent *E. coli* WM3064 stocks—prepared by employing a protocol described by Wu et al. (2010)—in 1 mm cuvettes at a voltage of 1.8 kV, a capacitance of 25 μF, and a resistance of 200 Ω. After selecting for successful transformants, the resulting *E. coli* strains were grown overnight and plated on LB+DAP in a suspension with *S. oneidensis* Δ*mtrB*—after being washed of residual kanamycin. Each conjugation was allowed to proceed for 8 h at 30 °C, after which a new LB+kanamycin plate, without DAP, was streaked for single colonies of *S.* oneidensis. Successfully conjugated *S. oneidensis* colonies were confirmed *via* colony PCR and Sanger sequencing.

2.3 Iron(III) citrate reduction

For high-throughput characterization of our arsenic sensing strains, we quantified their capacities to reduce Fe(III) at concentrations of arsenite ranging from 0 to 500 μM. While the pathways responsible for electrode reduction and soluble iron reduction do not fully overlap, MtrB plays a crucial role in both pathways, making the quantification of iron reduction ideal for our purposes (Rosenbaum et al., 2010; Coursolle and Gralnick, 2012). In preparation for the assay, all strains were cultured aerobically overnight in LB at an arsenite concentration to be employed in the subsequent assay. After growing to an OD_{600} of 1, cultures were washed and resuspended to a final OD_{600} of 0.015 in minimal M4 media with 20 mM sodium lactate and 10 mM Fe(III) citrate. Inside an anaerobic chamber, 30 μL of each cell suspension was added to 270 μL of minimal M4 media (and arsenite, where appropriate) in the wells of sterile 96-well plates. After incubating for 12 h, 5 μL from each well were transferred into clean wells of a new 96-well

plate, along with 45 µL of 0.5 M HCl and 300 µL ferrozine reagent (Coursolle et al. 2010, Stookey, 1970). For quantification, the resulting mixture was measured spectrophotometrically at 562 nm. Defined Fe(II) standards at an appropriate range of concentrations were used to calibrate the assay.

2.4 BES construction and electrochemical techniques

We employed single-chambered glass electrochemical reactors in the construction and characterization of our bench-scale BES-based biosensor. Operating BESs as electrochemical half-cells, we employed a either a commercial potentiostat (VSP, Biologic USA, Knoxville, TN; CHI660B, CH Instruments, Austin, TX) or an open-source, microcontroller-based potentiostat (placed in a Faraday cage for electronic shielding; Friedman et al., 2012a) to control and monitor electrochemical conditions in a three electrode system. We controlled the potential of a graphite working electrode (Poco Graphite, Inc., Decatur, TX) at 0.3 V *versus* a Ag/AgCl, saturated KCl glass reference electrode, while a graphite counter electrode served as a current drain. In this arrangement, *S. oneidensis* biofilm formation and electron transfer is induced at the working electrode, since it is poised at a potential that is energetically favorable for electrode reduction.

In continuous flow experiments, the working volume was held constant at 120 mL while media was fed *via* a peristaltic pump (Cole-Parmer, Vernon Hills, IL) at a continuous rate of 18 mL/h to maintain a hydraulic retention time of about 7 h. Because recent evidence suggests that oxygen intrusion allows *S. oneidensis* to overcome redox mediator washout in continuous-flow reactors (TerAvest, unpublished data), the head space of the reactor was left open to atmosphere

through a 0.2 μm filter (Pall Corporation, Cortland, NY) to maintain a micro-aerobic environment. This environment also provided the sensing strains with oxygen as an alternative to the electrode as an electron acceptor, so that electron acceptors would not be limiting when arsenic was not present (*i.e.*, in conditions where the Mtr pathway was incomplete).

We operated reactors for continuous-flow investigations at an initial arsenite concentration of zero. Current was recorded *versus* time for BESs inoculated with a *S. oneidensis* strain carrying pArsR/MtrB_1, as well as with Δ*mtrB* and MR-1 empty vector controls. We added kanamycin to the reactor feed to maintain a selective pressure for plasmid maintenance. After observing stationary current (with fluctuations within 5% of average value) for more than two hydraulic retention times, arsenite was added directly to a feed tank such that the steady-state arsenite concentration inside the reactors could be controlled. These steady-state arsenite concentrations were incrementally increased (again upon observation of stationary current) until a final concentration of 500 μM was reached.

We also performed batch investigations of a *S. oneidensis* strain carrying pArsR/MtrB_2, alongside as Δ*mtrB* and MR-1 empty vector controls. *S. oneidensis* strains were grown overnight in LB+kanamycin (without arsenite) to an OD_{600} just under 1.0 before being resuspended in minimal M4 (with kanamycin) to an OD_{600} of 0.015. Suspensions were inoculated into 100 μL of minimal M4 media with kanamycin and arsenite (when appropriate) after at least 5 h of abiotic background current was observed. While current was recorded over the entire length of batch experiments, peak current was of particular interest as a response variable to arsenite concentration.

3. RESULTS AND DISCUSSION

3.1 Design of an arsenic-sensing genetic circuit

We relied on an *mtrB* complementation strategy to construct arsenic-responsive genetic circuits in plasmids pArsR/MtrB_1 and pArsR/MtrB_2. MtrB plays an essential role in the metal reduction (Mtr) pathway of *Shewanella* spp., stabilizing a complex formed between MtrA and MtrC (Coursolle et al., 2010). Because this stable complex is required for electrode reduction, strains deficient in the *mtrB* coding sequence are unable to produce significant levels of current when inoculated into BESs. However, when *mtrB* is re-introduced into a knockout strain (and transcription is induced), the electrode reduction phenotype is restored—*i.e.*, when inoculated into an BES, current production will increase in response to increasing *mtrB* transcription in such an engineered strain. We exploited this by placing the *mtrB* coding sequence under the control of an arsenic-inducible promoter region (the molecular details of which are discussed in Section 2.2).

The arsenic-inducible promoter (P_{ars}) is negatively regulated by ArsR (Fig. 1). When arsenic is excluded from a cell with these genetic components, the binding kinetics between ArsR and its associated operator region within P_{ars} are relatively strong, blocking the transcription of downstream genes (*i.e., arsR* and *mtrB*). In this arrangement, ArsR acts as a negative auto-regulator by limiting its own expression to a low, basal level. The dynamics of the circuit change, however, when arsenic (in the form of arsenates or arsenites) enters the cell. In this condition, arsenic associates with ArsR, inducing a conformational change that leads to its dissociation from the P_{ars} operator. Because the binding kinetics between ArsR and the P_{ars}

operator become more unfavorable (to a point) with increasing arsenic concentrations, MtrB levels—and hence, electrode reduction capacities—increase with increasing arsenic presence.

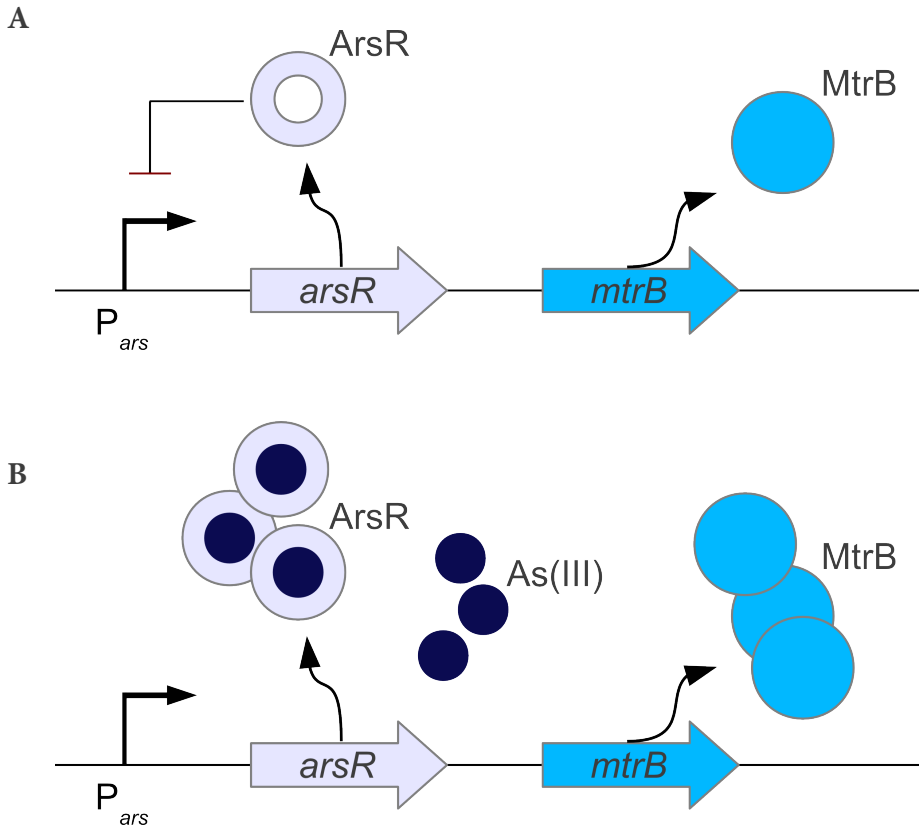

Fig. 1. Design of arsenic-responsive transcriptional circuit in in plasmids pArsR/MtrB_1 and pArsR/MtrB_2. *arsR* is a negative auto-regulator, inhibiting the transcription of itself and downstream *mtrB*. (**A**) When relatively little arsenic is present, ArsR is able to bind to the operator of P_{ars}, the arsenic-sensitive promoter. (**B**) As more arsenic enters the cell, ArsR dissociates from the operator, leading to an increase in *mtrB* expression.

3.2 Effect of arsenic on iron reduction

S. oneidensis strains carrying arsenic-responsive plasmids had increasing capacity to reduce Fe(III) to Fe(II) at increasing arsenite concentrations (Fig. 2). While a statistically significant change in iron reduction capacity was only observed at concentrations above 100 μM for the

strain carrying pArsR/MtrB_1, the strain carrying pArsR/MtrB_2 was able to reduce significantly more Fe(III) at arsenite levels below 80 µM—compared to basal iron reduction. This indicates that the efficiency of MtrB translation plays a role in determining arsenic sensitivity, as further described in Section 3.4. However, because *both* arsenic-sensing strains were able to reduce more iron at increasing arsenite concentrations, we have strong evidence that each strain can function as the biological component of an arsenic sensor with direct electronic output when inoculated into an BES.

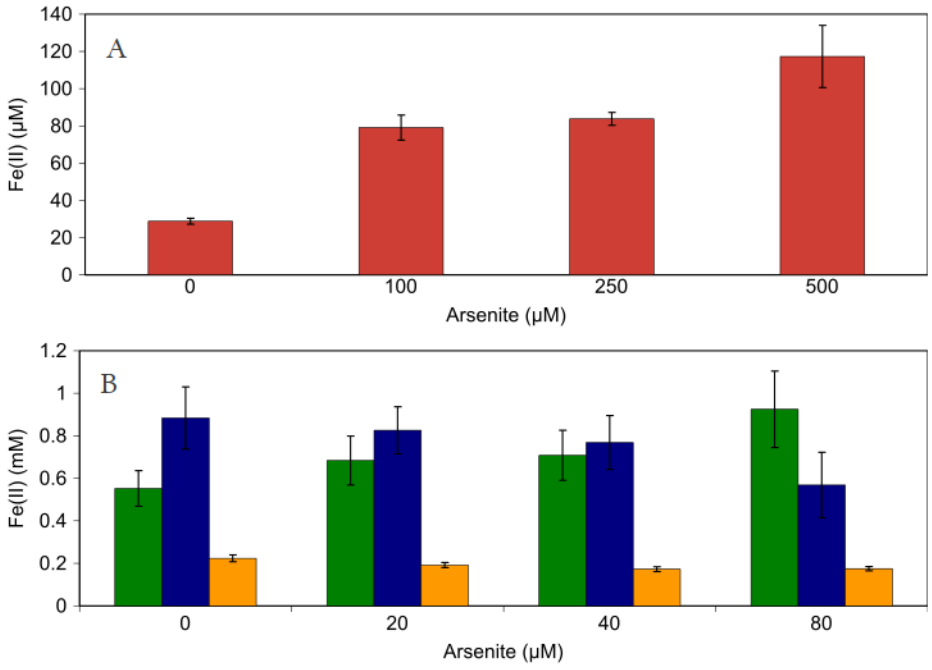

Fig. 2. Fe(II) evolution by *S. oneidensis* strains over 12 h at various concentrations of arsenite. (**A**) A significant improvement in iron reduction is observed for a *S. oneidensis* strain carrying pArsR/MtrB_1 at arsenite concentrations above 100 µM. (**B**) A wild-type positive control (blue) and a Δ*mtrB* negative control (orange) each show toxic responses to arsenic, as iron reduction capacity is diminished with increasing arsenite concentration; a *S. oneidensis* strain carrying pArsR/MtrB_2 (green) shows an increasing capacity to reduce iron at increasing arsenite concentrations, with significantly different Fe(II) evolution at 80 µM arsenite.

It is interesting to note that increasing MtrB expression can compensate for arsenic toxicity in our sensing strains—in terms of respiration rates. Both wild-type *S. oneidensis* MR-1 and the Δ*mtrB* negative control showed decreasing iron reduction rates at increasing arsenite concentrations (Fig. 2B), indicating that toxic effects of arsenite cause a growth defect in *S. oneidensis* strains at the range of concentrations under investigation. Therefore, it is likely that increasing MtrB expression "makes up" for this defect in our sensing strains. At arsenite concentrations above 100 μM, however, the *S. oneidensis* strain carrying plasmid pArsR/MtrB_2 shows diminished capacity to reduce iron (data not shown). This phenomenon may be explained by saturation in MtrB activity (disallowing compensation for arsenic toxicity) combined with the inherent toxic effects of overexpression of Mtr proteins—as recently investigated by Goldbeck et al. (2013).

3.3 Effect of arsenic on continuous flow BES current production

When inoculated into a continuous flow BES, the *S. oneidensis* strain carrying plasmid pArsR/MtrB_1 reproducibly generated increased steady-state current in response to increasing steady-state arsenite concentrations. An arsenite concentration of 500 μM induced a steady-state current of approximately 150% of the basal level (*i.e.*, current produced in an BES without arsenic; Fig. 3). While arsenite concentrations on the order of 100 μM induced a transient increase in current, an elevated steady-state current level was not achieved. While a definitive explanation for this phenomenon has yet to be tested experimentally, we hypothesize that changing dynamics in the menaquinone pool (which supplies the Mtr pathway

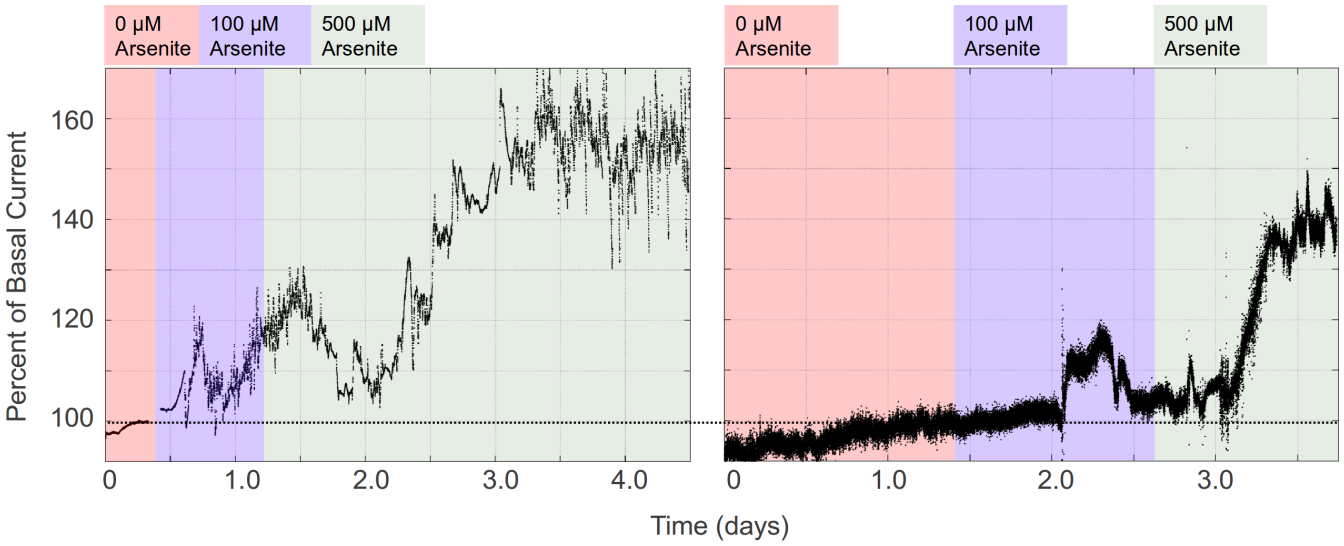

Fig. 3. Response of *S. oneidensis* strain carrying pArsR/MtrB_1 to arsenite in two replicates. After panning through a steady-state arsenite concentration of 100 µM, a final response was observed at 500 µM arsenite. After a transient phase on the order of a day, a new steady-state—about 50% above baseline current—was achieved.

with reducing equivalents to be shuttled outside of the cell as current) accounts for this transient behavior. Despite these complications, we have shown that steady-state current may be recorded as an indicator of arsenite concentration, forming the basis of our BES-based biosensor.

However, limitations of these sensing capabilities include a relatively long response time (on the order of 1 day). While it is possible that the biofilm turnover rate limits the ability of our biosensor to respond rapidly to changing arsenite concentrations, some factors contributing to response time are unknown. Because the engineered *S. oneidensis* strain is deficient in MtrB at low levels of arsenic, it is also possible that changes in transcriptional regulation (in multiple stages) are required before a fully functional Mtr pathway is able to form, further contributing to the time required to reach a new steady-state upon arsenic addition. Further investigation is required, however, to confirm the role of transcriptional regulation in the response time of the

biosensor. Such investigation, along with variation of the macro-scale parameters of the reactor (*e.g.*, biofilm shear stress due to the flow of media) will lead to improved response time in future BES-based biosensors relying on engineered *S. oneidensis* strains.

3.4 Electrochemical characterization of alternative sensing strain

In addition to the relatively long response time of the continuous flow BES-based biosensor, the detection limit on the order of hundreds of μM arsenite is also of concern. For practical applications, such a biosensor would need to be much more sensitive, because both the USEPA and WHO have set standards for maximum chronic exposure to arsenic salts that are orders of magnitude below the detection limit of the *S. oneidensis* strain carrying plasmid pArsR/MtrB_1 (McGuigan et al., 2010). Consequently, the arsenic-responsive circuit in pArsR/MtrB_1 requires modification to increase arsenic sensitivity.

As mentioned in Section 2.2, the *mtrB* upstream sequence was modified in the insertion of a BamHI cut site in pArsR/MtrB_1. The nucleotide changes in this altered upstream sequence are shown in Table 1. Because KineFold (http://kinefold.curie.fr/) simulations predict

	***mtrB* Upstream Sequence**
Native	**AGGAGA**AATACTAG*ATG*
Altered	A**AGGAGA**CGGATCC*ATG*

Table 1. *mtrB* upstream sequences for native (wild type and pArsR/MtrB_2) and altered (pArsR/MtrB_1) genotypes. During the construction of pArsR/MtrB_1, the number and identity of spacer nucleotides flanked by the ribosomal binding site (RBS; bold) and the start codon (italic) were changed upon introduction of a BamHI cut site (underlined).

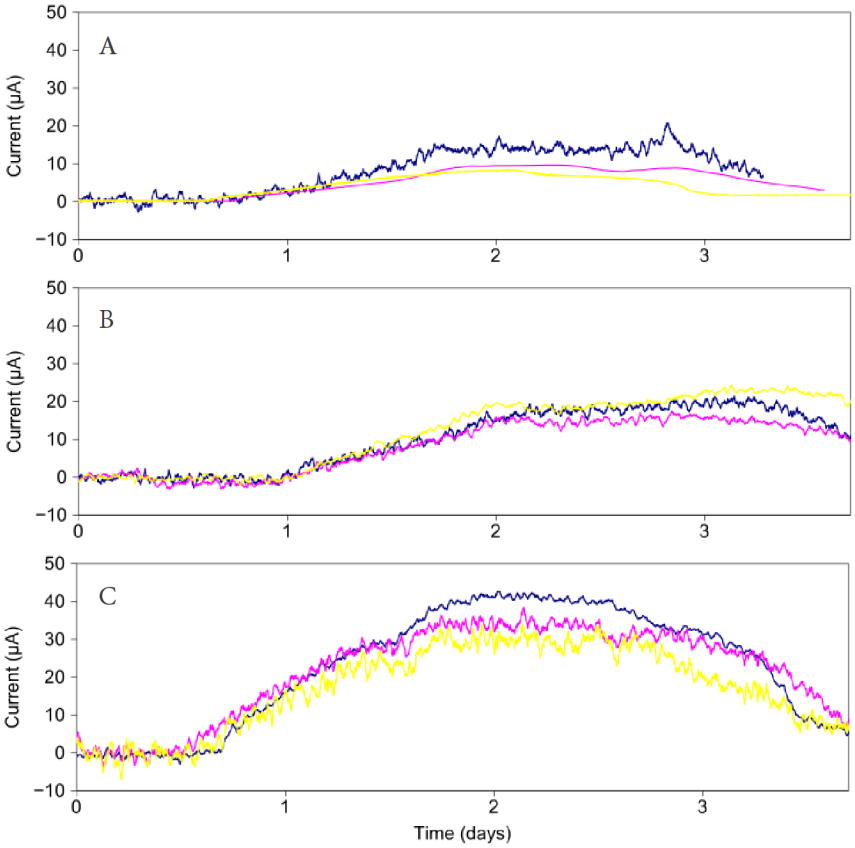

Fig. 4. Current production over time for *S. oneidensis* strain carrying plasmid pArsR/MtrB_1 in batch BESs. Three replicates are shown in each panel. When inoculated into a reactor without arsenic (**A**), maximum current production is consistently below 20 μA for this engineered strain; in reactors with 40 μM arsenite (**B**), maximum current is around 20 μA; in reactors with 100 μM arsenite (**C**), maximum current is above 30 μA.

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mRNA folding to partially block access to the RBS of *mtrB* for this sequence, it is likely that strains carrying pArsR/MtrB_1 exhibit reduced MtrB translational efficiency. As mentioned in Section 2.2, however, pArsR/MtrB_2 retained the native *mtrB* upstream sequence; strains carrying this plasmid should not exhibit such reduction. This was partially confirmed by the results of iron reduction assays presented in Section 3.2, since strains carrying pArsR/MtrB_2 displayed a significantly higher capacity to reduce iron at relatively low arsenite concentrations—

suggesting that higher MtrB concentrations were achieved. Further evidence for the enhancement in translational efficiency conferred by pArsR/MtrB_2 is shown in Fig. 4 above, which displays current production in batch BESs at three arsenite concentrations.

While further controls and replicates are required to achieve statistically significant results, preliminary evidence makes it clear that the *S. oneidensis* strain carrying pArsR/MtrB_2 is able to respond to arsenic concentrations on the order of tens of μM, a full order of magnitude less than the detection limit of the original strain. While further work is required to improve the detection limit of our BES-based biosensor, it is encouraging that modulation of translational efficiency is an apparently effective approach in the tuning of biosensor sensitivity. Because the construction of RBS libraries (characterized in specific hosts) is already underway as part of the Registry of Standard Biological Parts, this implies that synthetic circuits with modulated sensitivity will be relatively easy to build as the burgeoning field of synthetic biology expands.

4. CONCLUSIONS

We have shown that it is possible to transduce chemical information—of relevance to environmental quality—into the digital world by employing a BES inoculated with genetically engineered *S. oneidensis*. By characterizing current responses of our engineered strains to arsenic, we have moved beyond a proof of principle, and have constructed an BES-based biosensor with direct environmental applications. Because our biosensor outputs current directly, it may be more easily integrated into a deployable device for remote monitoring than more traditional microbial biosensors. Such a prototype (designed for the remote monitoring of arsenic in natural

bodies of water) was constructed as part of the 2012 International Genetically Engineered Machine (iGEM) Competition by Cornell iGEM (http://2012.igem.org/Team:Cornell; Fig. 5). Briefly, this device is capable of supporting monitoring for up to six

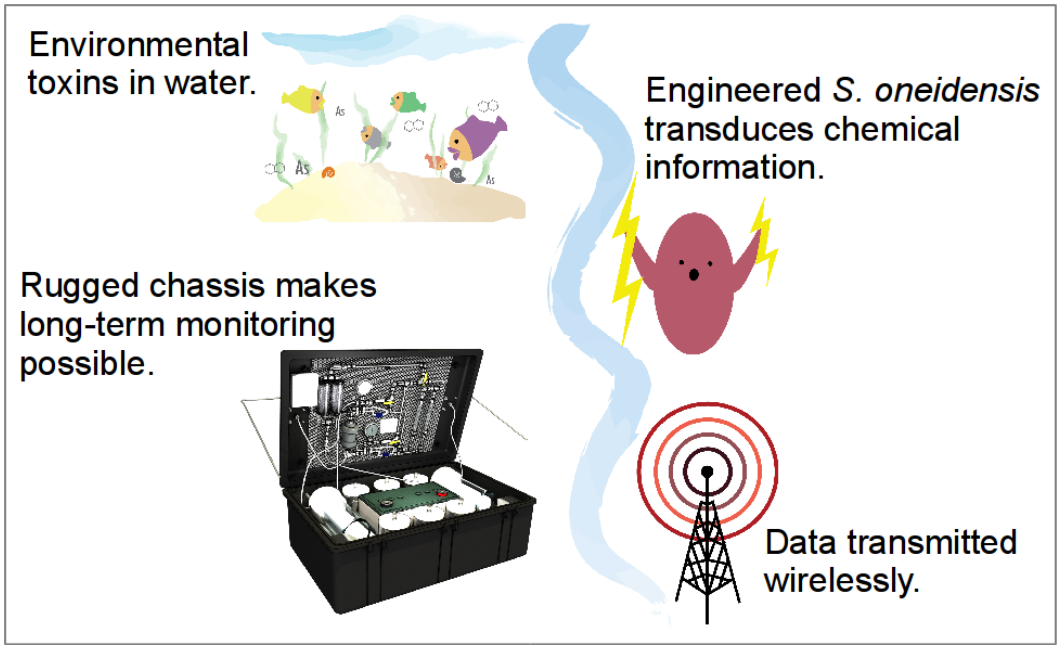

Fig. 5. Overview of field-deployable biosensor developed for 2012 iGEM competition. An engineered, arsenic-responsive *S. oneidensis* strain is inoculated into a reactor housed in a rugged device—alongside a control strain to account for fluctuations in environmental parameters. Sterile lactate is fed to reactors from vessels built into the device, combining with a filter-sterilized sample stream. On-board electronics record current over time, and transmit data wirelessly so that arsenic concentrations can be monitored remotely.

months—maintaining a continuous culture of an arsenic-sensing *S. oneidensis* strain with a concentrated stock of substrate—and is capable of wirelessly transmitting water quality data by employing a modified version of a previously published open-source potentiostat (Friedman et al., 2012a). Because such a device is less expensive and labor intensive than more conventional analytic chemistry approaches, it is clear that BES-based biosensors are ideal for deployment as a

first-pass monitoring system for remote detection of contamination events. As Golitsch et al. (2013) note, because *S. oneidensis* strains can be modified to detect a multitude of analytes of environmental interest with the simple swap of a promoter, a number BES-based biosensors may be constructed by hijacking the regulation of the Mtr pathway of *S. oneidensis*. Because of this, along with the fact that the genetic system of this organism is well worked-out, we believe *S. oneidensis* to be an ideal host for development of biosensors for remote monitoring of environmental contaminants.

5. APPENDIX A. SUPPORTING INFORMATION

Primer	Restriction Site(s)	Sequence
MtrB_1	AscI/BamHI	TTCTTCGGCGCGCCTAAGGAGACGGATCCATGAAATTTAAACTCAATTTGATCACTC
MtrB_2	KpnI/SpeI/PstI	GTTTCTTCCTGCAGTCAGACGAACTAGTAGGTACCATAGAAGGTGAGCCAGTGTGAC
ArsR_1	EcoRI	CGTATCACGAGGCAGAATTC
ArsR_2	AscI/SpeI/PstI	GTTTCTTCCTGCAGTACTAGTAGGCGCGCCGTTAACTGCAAATGTTCTTACTGTCC

Table S1. Primers and restriction sites used for cloning and subsequent generation of arsenic-sensing strains. MtrB_1 and MtrB_2 were used to amplify *mtrB* from BBa_K098994 (Registry of Standard Biological Parts); ArsR_1 and ArsR_2 were used to amplify *arsR* from BBa_J33201 (Registry of Standard Biological Parts).

5. 1 GenBank Formatted Sequence for Plasmid pArsR/MtrB_1:

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LOCUS       p_arsR/mtrB_p14        6525 bp    DNA     circular UNA 20-MAR-2013
DEFINITION  Arsenic inducible expression of mtrB; Derived from pBBRBB-eGFP.
ACCESSION   urn.local...1363788224712.13
VERSION     urn.local...1363788224712.13
KEYWORDS    .
SOURCE
  ORGANISM
            .
FEATURES             Location/Qualifiers
     misc_feature    complement(<1..305)
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                     /label="Rep"
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                     GAVELHDGDPALGHQVLDCVLDRPQRTSDELGKCLLADHHGVLVAHLRHEVMQQHCRR
                     GFPRNKPGPRLMRFAFRLHPVTGLVLGLNADFSGLRGHAYLHAVGCRTVAAPCAISCN
                     FSAARQQLCVA*"
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